The acaricidal effect of ethanolic extracts of *Chenopodium quinoa* Willd. on *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Ethanolic extracts obtained from the seed coat of *Chenopodium quinoa* Willd. were evaluated for their acaricidal effect on different stages of the carmine spider mite *Tetranychus urticae* Koch. At 72 h after application of the extracts directly on the adult females, mortalities ranged from 30 % to 93 %, with concentrations of 6.11, 7.61 and 9.11 % w/v producing the best results. The lethal effect of the extracts on the nymphs of *T. urticae* manifested at 24 h after inoculation, with mortality rates ranging between 50 % and 99 %. The extracts of *C. quinoa* had no lethal effect on the eggs of *T. urticae*. At 120 h post-application, between 76 % and 89 % of the larvae had hatched. The extracts showed a repellent effect on adult females of *T. urticae*. Oviposition by female *T. urticae* was not affected by application of sublethal concentrations of the extract. The survival of juveniles whose mothers were sprayed with the extracts ranged between 17 % and 48 %, showing a sublethal effect of the extracts on the offspring. Lethal concentrations of the extract of *C. quinoa* for adult females of *T. urticae* were determined as 1.24 % w/v (LC_{50}) and 4.34 % w/v (LC_{90}).

Key words: lethal and sublethal effects, repellency, irritancy, LC_{50}, LC_{90}, *Tetranychus urticae.*

INTRODUCTION

The carmine form of the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), formerly known as *Tetranychus cinnabarinus* (Boisduval) (Auger *et al*. 2013), is one of the most important pests of crops and ornamental plants worldwide (Capinera 2001). In Chile it is widely distributed and its hosts include *Cucumis melo* (L.), *Phaseolus vulgaris* (*Fragaria* × *ananassa* (Duch.) and *Dianthus caryophyllus* (L.) (Klein & Waterhouse 2000).

For several decades the control of *T. urticae* has relied mainly on pesticide applications. The intensive use of pesticides has resulted in the rapid development of resistance in this mite (Van Leeuwen *et al.* 2010; Wei *et al*. 2011) and a high demand for new acaricides. Thus many researchers are currently trying to obtain useful compounds from plants to produce new natural pesticides. An interesting class of molecules in this regard is the saponins, a group of steroid or triterpenoid secondary metabolites with different biological activities (De Geyter *et al*. 2007).

Some saponins have an antifeedant activity, as those extracted from *Ilex apocea* that inhibit feeding of *Lymantria dispar* (Barbosa *et al*. 1990). These saponins also have antifeedant effects on the spider mite *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) and two species of caterpillars, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) and *Malacosoma americanum* (Fabricius) (Lepidoptera: Lasiocampidae) (Kreuger & Potter 1994; Oleszek *et al*. 1999).

Saponins from *Chenopodium quinoa* Willd. (Chenopodiacae) have shown insecticidal, antibiotic, fungicidal and pharmacological action (Abugoch 2009). Woldemichael & Wink (2001) demonstrated antifungal activity of these saponins against the human pathogen *Candida albicans* (CP Robin) Berk. Stuardo & San Martin (2008) found antifungal activity of the quinoa saponins treated with alkali against *Botrytis cinerea* Pers., while Iannacone & Quispe (2004) found insecticidal activity of chloroform extracts of quinoa seed on the weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae).

In view of the biological activity of the quinoa saponins against various organisms, this study was undertaken to evaluate the acaricidal effect of ethanolic extracts from seeds of *C. quinoa* coming from the highlands of northern Chile against *T. urticae*.

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MATERIAL AND METHODS

Assays were conducted in the Laboratory of Plant Health of Universidad Arturo Prat, located in the Canchones Experimental Station, Tamarugal Province, Tarapaca Region, Chile (20°16′ 15.3″S 70°07′46.6″W). Experimental conditions of 26 ± 2 °C, 70 ± 10 % RH and a photoperiod of 16:8 (light:dark) were used throughout.

Quinoa seeds

The seeds of *C. quinoa*came from plants grown in the last harvest during April 2013 in the town of Colchane, Tarapacá Region, Chile [19°16′ S, 68°38′ W; 3 715 m a.s.l. (metres above sea level)]. Ethanolic extracts were obtained at the Laboratory of Natural Products, Faculty of Health Sciences, Arturo Prat University, Playa Brava Campus, Iquique, Chile.

Preparation of ethanol extract

After scarification of quinoa grain, the powder obtained was subjected to a process of continuous extraction through a Soxhlet (Brand Fisatom, Class 650 model 23, Brazil). Approximately 120 g of powder was weighed and placed in a closed filter wrapper paper. The Soxhlet capacity of 750 ml was refluxed with 1.5 l of ethanol for 24 h. After the extraction, it was filtered and the solvent evaporated in a rotary evaporator (Heidolph brand, Laborota 4001 model, Germany) at low pressure and temperature, thereby obtaining a crude ethanolic extract of the quinoa grain. This was stored in amber bottles in the absence of light at –20 °C.

Collecting and rearing Tetranychus urticae

Tetranychus urticae was collected from carnation plants (*Dianthus caryophyllus* L.) in the Chintaguay agricultural community, La Tirana, Tamarugal Province, Tarapaca Region, Chile (20°21′S 69°39′W; 1004 m a.s.l). Infested leaves were placed in Petri dishes and transferred to the laboratory where the mites were further reared on bean plants (*Phaseolus vulgaris* L. variety 'canary').

A constant supply of bean leaves infested with *T. urticae* was required for the bioassays. This was obtained by planting 100 to 200 bean seeds weekly in pots with organic soil and infesting them with the spider mites collected. Mites were reared in the laboratory for several generations before they were used in bioassays. Bean plants were kept in the Laboratory of Plant Protection at 27 ± 2 °C, 50–70 % RH and photoperiod of 16:8 light:dark (Vargas *et al*. 2002), and irrigated every two days; senescent plants were replaced by new ones.

Bioassays

Preliminary tests were carried in order to establish the concentrations to be used in evaluating the effect of the ethanolic extracts by direct application of the extract on the adults and eggs of *T. urticae* and by indirect applications. Exploratory concentrations of the extracts tested were 0.5 %, 1.5 %, 5 % and 10 % w/v and the concentrations finally applied 0.1 %, 1.6 %, 3.1 %, 4.6 %, 6.1 %, 7.6 % and 9.1 % w/v, unless otherwise stated. Each treatment was sprayed with 2 ml of each concentration (Kabir & Chapman 1997) using a Potter tower (Burkard Manufacturing Co. Ltd., Rickmansworth, England) with a working pressure of 55 kPa and a time of 5 s. The distance between the spray nozzle and the leaves was 50 cm. Under these conditions the spray deposit was calculated as 1.25 ± 0.01 mg/cm^2 (Bowie *et al*. 2001). The ambient conditions were the same for all trials, *i.e*. 26 ± 2 °C, 70 ± 10 % RH and a photoperiod of 16:8 L:D (Vargas *et al*. 2002). The observations were made with a stereomicroscope Carl Zeiss Stemi SV 6. The temperature and relative humidity were recorded with a data logger (Dickson, model MP-1000). Specimens were considered dead if appendages did not move when they were pricked with a fine brush (Miresmailli *et al*. 2006) or if the mites were unable to walk at least one body length when they were weakly stimulated with a fine brush (Kabir & Chapman 1997).

Toxicity tests on adults by direct contact

Eggs produced by approximately 50 females during a period of 12 h were collected in plastic cages. The cages containing the eggs were kept at the same condition to hatch. This cohort was bred until the adults were obtained. Subsequently, groups of 10 adult female *T. urticae* were selected at random and placed on leaf discs 2 cm in diameter. The leaves were previously washed with distilled water and bordered with glue (Point StickyGlue®) to prevent the mites escaping. The leaf discs were placed on soaked cotton in plastic Petri dishes (9 cm × 1.5 cm) and the treatment applied. The experiment was repeated 10 times for each of the seven concentrations. Three control treatments were used: ethanol (99.8 %), abamectin; a synthetic acaricide (Fast

Plus®) applied at the recommended dose 70 ml per 100 l of water (0.036 mg of abamectin per application), and no application (to correct for mortality by handling). Mortality was determined at 24, 48 and 72 h after treatment (Sivira *et al*. 2011).

Toxicity tests on nymphs by direct contact

Nymphs (2 days old) were obtained by introducing five adult female of *T. urticae* on leaf discs and allowing them to oviposit for 24 h. Females were removed when at least 20 eggs per disc were obtained. After about 7 days the larvae hatched, as was also found by Nauen *et al*. (2001). The experimental unit was a group of 10 protonymphs and each treatment was repeated 10 times. Mortality was valued at 24, 48 and 72 h (Sivira *et al*. 2011).

Toxicity test on eggs by direct contact

Bean leaf discs were used as substrate for oviposition. Three adult females randomly selected were transferred with a fine brush to each disk, allowing oviposition for 24 h (Afifi *et al*. 2012; Duso *et al*. 2008; Tsolakis & Ragusa 2008). They were always accompanied by a male to assure the fertilisation of eggs and avoid pseudo-arrhenotokous parthenogenesis. Mites were removed when at least 10 eggs per disc were obtained. The experimental unit was a group of 10 eggs and each treatment was repeated 10 times. Hatched larvae were counted daily until the seventh day, since the incubation period for this mite ranges between 4 and 6 days (Tello *et al*. 2009; Peralta & Tello 2011; Kazak & Kibritci 2008) or when the controls (except acaricide) had 90 % hatched larvae (Lim *et al*. 2012).

Toxicity test on adults by residual contact

Bean leaf disks of 2 cm diameter were immersed in each extract concentration (6.1 %, 7.6 % and 9. 1% w/v) in 2 ml of solution for 30 s (Jeon *et al*. 2010). Excess liquid was removed and the disks were dried at room temperature (25 °C for 0.5 h) (Sivira *et al*. 2011; Erdogan *et al*. 2012). The leaf discs were bordered with glue (Vargas *et al*. 2002) to prevent mite escape and placed on moist cotton in Petri dishes (Sivira *et al*. 2011). Finally, using a fine brush, 10 mites were transferred to the surface of each disc (Abo-Moch *et al*. 2010). As a control, the solvent ethanol (99.8 %) was used. Each experimental unit consisted of a disc with 10 individuals and each treatment was repeated 10 times. The treatments were 6.1, 7.6 and 9.1 % w/v.

Lethal concentrations ($LC_{50,90}$) and lethal time (LT_{50})

Adult females of *T. urticae* were sprayed for each extract concentration (0 %, 1.5 %, 3 %, 4.5 %, 6 %, 7.5 %, 9 %, 10.5 % w/v) with 2 ml of solution using a Potter tower (Kabir & Chapman 1997). For each trial, the experimental unit consisted of a disc with 10 individuals and each treatment was repeated 10 times. To calculate the LT_{50}, mortality was evaluated every 12 h (methodology adapted from Flores *et al*. 2007; Shi *et al*. 2008; Bugeme *et al*. 2009; Motazedian *et al*. 2012) until the third day (Sivira *et al*. 2011).

Repellency test

For each application 10 leaf discs of 4 m diameter were cut, leaving the midrib to divide the surface, and placed in a Petri dish. The outer surface half of each disc was covered with a thin sheet of aluminum foil (Alusa®) and 2 ml of extract of *C. quinoa* was applied on the discs using a Potter tower. The extract concentration applied was determined through preliminary tests and 2 %, 3 % and 4 % w/v were selected. An application of ethanol (99.8 %) served as a control. The experimental unit was a group of 10 adult females. After the application the sheet of aluminum foil was removed and the leaf discs allowed to dry for 5 min at room temperature. Each leaf disc was bordered with glue. Ten adult mites were placed in the leaf disc area which was covered with aluminum foil and received no treatment. The repellent effect was determined by calculating the percentage of adults who did not move to the treated area (Hussein *et al*. 2006) at three assessment periods of 24, 48 and 72 h after treatment (Sivira *et al*. 2011).

Oviposition test

Adult females were sprayed in a Potter tower with extract concentrations of 2 %, 3 % and 4 % w/v and 99 % ethanol as a control. Subsequently a male and one of the sprayed females were placed on an unsprayed leaf disc of 3 m diameter [methodology adapted from Attia *et al*. (2011) and Saenz de Cabezon *et al*. (2002)]. This was repeated 10 times. Two parameters were evaluated: a) number of eggs laid per female and b) percentage of hatched nymphs that reached the adult stage. Oviposition was recorded until the third day post application. For case b), the hatched larvae were monitored (after removing the adults) on the same leaf discs until they reached the adult stage. This took about

15 days (Kazak & Kibritci 2008; Tello *et al*. 2009; Peralta & Tello 2011).

Design and statistical analysis

A completely randomised design was used for all tests. The control mortalities were corrected by using Abbott's formula (Abbott 1925) and the data were normalised by angular transformation (arcs √%/100). Subsequently, an analysis of variance was performed and means were separated using Tukey's test. For comparisons of two means, Student's *t*-test was used. The Shapiro-Wilks and Levene tests were used to ensure normality and homogeneity of variance, respectively.

Lethal concentrations and times

In order to calculate the $LC_{50,\ 90}$ and TL_{50}, the results were processed using probit analysis (Finney 1971), following the methodology used by Salazar & Araya (2001). Data were analysed using Statistical Analysis System 9.1 (SAS Institute, Cary, NC, U.S.A.) and dose-mortality regressions were estimated by probit analysis (PROC PROBIT).

RESULTS

Toxicity test on adults by direct contact

Mortalities for *T. urticae* adults at the different extract concentrations, evaluated at 24, 48 and 72 h, are shown in Table 1. Significant differences were detected between treatments at 24 h ($F_{(9,85)} = 77.96$, $P < 0.001$), 48 h ($F_{(9,84)} = 44.43$, $P < 0.001$) and 72 h ($F_{(9,85)} = 33.17$, $P < 0.001$). The abamectin (chemical control) application resulted in 100 % mortality at the three evaluated times. The concentrations that showed a statistically similar response to abamectin were 9.1 % w/v at 48 h; 7.6 % and 9.1 % w/v at 72 h. In this case, the increase in the concentration also resulted in an increase in the mortality of the adults. All concentrations applied had a significantly increased mortality at the three times of evaluation. The ethanol application did not kill any mites.

Mortality of adult females of *T. urticae* after indirect application

Mortality of *T. urticae* adults after indirect application is shown in Table 2. There were significant differences between treatments at all evaluation periods (24 h: $F_{(5,51)} = 33.54$, $P < 0.001$, 48 h: $F_{(5,50)} = 47.43$, $P < 0.001$ and 72 h: $F_{(5,43)} = 53.45$, $P < 0.001$). At 48 h, the 7.6 % and 9.1 % w/v treatments showed greater than 50 % mortality. After 72 h these concentrations were unique in being statistically similar to chemical control, which reached 100 % mortality throughout the test.

Mortality of nymphs of *T. urticae* after direct application

The results of the mortality in nymphs of *T. urticae* for each extract concentration evaluated at 24, 48 and 72 h are indicated in Table 3. There were significant differences between treatments at 24 h ($F_{(9,90)} = 78.14$, $P < 0.001$), 48 h ($F_{(9,90)} = 123.08$, $P < 0.0001$) and 72 h ($F_{(9,90)} = 161.10$, $P < 0.0001$). Abamectin (chemical control) produced mortalities of 100 % at the three evaluation times. The concentrations that had a statistically similar response to abamectin, exhibiting a high acaricidal activity, were at 4.6 %, 6.1 %, 7.6 % and 9.1 % w/v at 24 h;

Table 1. Mortality (%) in adult females of *Tetranychus urticae* after direct application of *Chenopodium quinoa* extract.

Concentration (% w/v)	*n*	Average mortality[2] (± S.E.[3])		
		24 h	48 h	72 h
Ethanol[1]	82	0.00 ± 0.00 a A	0.00 ± 0.00 a A	0.00 ± 0.00 a A
0.1	88	13.25 ± 2.97 bc A	20.75 ± 5.91 bc AB	30.03 ± 5.95 bc B
1.6	87	18.41 ± 5.08 c A	30.84 ± 7.01 c AB	45.44 ± 8.69 cd B
3.1	95	23.20 ± 4.76 cd A	45.74 ± 7.04 cd AB	60.71 ± 9.29 cdf B
4.6	84	43.45 ± 4.15 df A	59.89 ± 6.27 d AB	69.23 ± 8.25 dfg B
6.1	98	45.97 ± 5.33 f A	69.78 ± 5.71 df B	83.50 ± 4.54 fgh B
7.6	98	57.37 ± 4.13 fg A	73.20 ± 5.38 df AB	83.13 ± 4.26 fgh B
9.1	93	73.10 ± 5.00 g A	87.20 ± 3.8 fg AB	92.77 ± 3.12 gh B
Abamectin 1.8	100	100.0 ± 0.00 h A	100.0 ± 0.00 g A	100.0 ± 0.00 h A

[1]Means with different lowercase letters within each column are statistically different according to the Tukey test ($P < 0.05$).
[2]Means with different uppercase letters in the same row are statistically different according to the Tukey test ($P < 0.05$).
[3]S.E.: standard error.

Table 2. Mortality (%) of *Tetranychus urticae* adults by indirect application of different concentrations of *Chenopodium quinoa* extract.

Concentration (% m/v)	*n*	Average mortality[1] (± S.E.[2])		
		24 h	48 h	72 h
Ethanol	71	0.00 ± 0.00 a	0.00± 0.00 a	0.00 ± 0.00 a
6.1	70	20.21 ± 5.94 ab	45.46 ± 5.87 b	73.70 ± 9.12 b
7.6	52	13.41 ± 7.15 a	58.33 ± 10.08 bc	89.82 ± 5.29 bc
9.1	41	45.76 ± 13.20 b	77.75 ± 6.68 c	91.44 ± 5.73 bc
Abamectin 1.8	57	100.00 ± 0.00 c	100.00 ± 0.00 d	100.00 ± 0.00 c

[1]Means with different letters within each column are statistically different according to the Tukey test ($P < 0.05$).
[2]S.E.: standard error.

Table 3. Mortality (%) after direct application of an ethanolic extract of *Chenopodium* quinoa on nymphs of *Tetranychus urticae.*

Concentration (% p/v)	*n*	Average mortality[2] (± S.E.[3])		
		24 h	48 h	72 h
Ethanol[1]	96	3.47 ± 2.43 a A	7.81 ± 2.90 a A	10.19 ± 3.54 a A
0.11	94	60.75 ± 8.77 b A	77.03 ± 4.36 b AB	86.32 ± 3.23 b B
1.61	99	50.22 ± 7.36 bc A	79.33 ± 5.94 b B	89.44 ± 5.45 bc B
3.11	98	79.67 ± 5.76 cd A	90.89 ± 3.15 bc AB	97.00 ± 2.13 cd B
4.61	100	91.00 ± 2.33 df A	96.00 ± 1.63 c A	97.00 ± 1.53 cd A
6.11	100	94.00 ± 2.21 df A	98.00 ± 1.33 c A	99.00 ± 1.00 cd A
7.61	99	96.00 ± 1.63 df A	98.00 ± 1.33 c A	99.00 ± 1.00 cd A
9.11	99	99.00 ± 1.00 f A	100.0 ± 0.00 c A	100.0 ± 0.00 d A
Abamectin 1.8[1]	100	100.0 ± 0.00 f A	100.0 ± 0.00 c A	100.0 ± 0.00 d A

[1]Means with different lowercase letters within each column are statistically different according to the Tukey test ($P < 0.05$).
[2]Means with different uppercase letters in the same row are statistically different according to the Tukey test ($P < 0.05$).
[3]S.E.: standard error.

and 3.1 %, 4.6 %, 6.1 %, 7.6 %, and 9.1 % w/v at 72 h. As regards the residual effect, the three lower concentrations had a significant increase in mortality over time, while higher concentrations reached percentages of 90 % from 24 h.

Direct application on *T. urticae* eggs

The number of *T. urticae* larvae hatching after application on the eggs is shown in Table 3. No concentration was able to avoid larvae hatching. All hatched larvae of the eggs exposed to different concentrations of the extract were statistically similar to the ethanol control. The acaricide abamectin significantly reduced the hatching of larvae after 72 h.

Repellent effect

At 24 h, all three concentrations showed a strong repellent effect with high percentages of the mites remaining in the untreated part of the leaf disc (2 % w/v: $t_{(10)} = 13.09$, $P < 0.001$; 3 % w/v: $t_{(10)} = 23.41$; $P < 0.001$; 4 % w/v: $t_{(10)} = 1\,915.75$, $P < 0.001$) (Table 5). After 48 h more mites moved to the treated area, but the differences between the two areas of the leaf disc remained significant for the three concentrations of the extract (2 % w/v: $t_{(10)} = 8.16$, $P < 0.001$; 3 % w/v: $t_{(10)} = 5.67$, $P < 0.001$; 4 % w/v: $t_{(10)} = 5.67$, $P < 0.001$). After 72 h the percentage of individuals in the treated area was even more, but the differences remained significant (2 % w/v: $t_{(10)} = 5.81$, $P < 0.001$; 3 % w/v: $t_{(10)} = 5.98$, $P < 0.001$; 4 % w/v: $t_{(10)} = 3.29$, $P < 0.001$).

Effect on oviposition

The results of sublethal effects of the ethanolic extract of *C. quinoa* on fecundity, fertility and sex ratio of *T. urticae* are presented in Table 6. Regarding fecundity, all concentrations tested did not

Table 4. Larvae hatching (%) after direct application of an ethanolic extract of *Chenopodium quinoa* on eggs of *Tetranychus urticae*.

Concentration (% m/v)	*n*	Larvae hatched (% ± S.E.[2])				
		24 h	48 h	72 h	96 h	120 h
Ethanol	100	0.00 ± 0.00 a	4.00 ± 2.21 a	37.36 ± 6.89 a	81.89 ± 5.10 a	91.00 ± 4.33 a
0.1	100	0.00 ± 0.00 a	7.00 ± 3.35 a	55.22 ± 7.38 a	76.78 ± 6.15 a	88.89 ± 3.14 a
1.6	100	0.00 ± 0.00 a	1.00 ± 1.00 a	50.00 ± 8.03 a	73.00 ± 8.17 a	82.00 ± 7.42 a
3.1	100	0.00 ± 0.00 a	3.00 ± 3.00 a	48.00 ± 10.31 a	72.00 ± 9.98 a	78.00 ± 7.86 a
4.6	98	0.00 ± 0.00 a	5.25 ± 1.77 a	49.25 ± 8.50 a	65.75 ± 9.53 a	69.75 ± 10.09 a
6.1	100	0.00 ± 0.00 a	2.00 ± 1.33 a	31.00 ± 7.95 b	62.00 ± 8.67 a	82.00 ± 5.93 a
7.6	99	0.00 ± 0.00 a	0.00 ± 0.00 a	44.00 ± 11.50 a	63.78 ± 13.13 a	77.00 ± 10.75 a
9.1	99	0.00 ± 0.00 a	1.00 ± 1.00 a	41.00 ± 11.69 a	69.56 ± 9.13 a	75.89 ± 8.83 a
Abamectin 1.8	99	0.00 ± 0.00 a	1.00 ± 1.00 a	1.00 ± 1.00 b	1.00 ± 1.00 b	1.00 ± 1.00 b
F		1.00	1.86	4.14	8.57	13.44
P		0.4460	0.0686	0.0002	0.0001	0.0001

[1]Means with different letters within each column are statistically different according to the Tukey test ($P < 0.05$).
[2]S.E.: standard error.

differ significantly from the control at all measurement periods (24 h: $F_{(3,33)} = 1.10$, $P = 0.3622$; 48 h: $F_{(3,33)} = 0.72$, $P = 0.5451$; 72 h: $F_{(3,33)} = 0.75$, $P = 0.5283$) which implies that oviposition rates did not decrease when females were exposed to the extract concentrations and the number of eggs per female did not differ between treatments.

Regarding fertility, *i.e.* the percentage reaching adulthood, the results for the three concentrations tested were statistically similar to each other. Low percentages of larvae that reached adulthood, significantly different from those of the control ($F_{(3,33)} = 7.40$, $P = 0.001$) indicating that the ethanolic extract of *C. quinoa* affects the fertility of females.

Regarding the sex ratio, the concentration 3 % w/v ($t_{(16)} = 2.48$, $P < 0.05$) was the only one not significantly different from the control ($t_{(14)} = 2.54$; $P < 0.05$) which reflected the normal situation where there is a higher percentage of females than males. The situation was different for individuals treated with 2 % ($t_{(12)} = 0.37$, $P = 0.72$) and 4 % w/v ($t_{(10)} = -0.03$, $P = 0.98$) extract where there were no significant differences between the ratio of the sexes.

Concentrations ($LC_{50, 90}$) and lethal time ($LT_{50, 90}$)

The results of probit analysis showed that the concentrations of the ethanolic extract of *C. quinoa* which achieved mortalities of 50 % and 90 % of *T. urticae* were 1.24 % and 4.34 % w/v, respectively (Table 7).

Table 5. Repellent activity of *Chenopodium quinoa* extract evaluated on adult females of *Tetranychus urticae* at three evaluation periods.

Concentration	Percentage of adults of *T. urticae* on the untreated and treated part of the leaf					
	24 h		48 h		72 h	
	Treated part	Untreated part	Treated part	Untreated part	Treated part	Untreated part
	(% mean[1] ± S.E.[2])					
Ethanol	42.70 ± 5.66 a	57.31 ± 5.66 a	61.05 ± 4.04 a	38.95 ±4.04 b	61.27 ± 4.85 a	57.31 ± 5.66 a
2 %	3.36 ± 1.72 b	96.64 ± 1.72 a	6.98 ± 3. 83 b	93.02 ±3.83 a	13.31 ± 4.71 b	86.69 ± 4.71 a
3 %	1.00 ± 1.00 b	99.00 ± 1.00 a	11.36 ± 6.27 b	88.64 ±6.27 a	14.69 ± 3.85 b	85.31 ± 3.85 a
4 %	0.00 ± 0.00 b	100.00 ± 0.00 a	12.03 ± 5.88 b	87.97 ±5.88 a	23.76 ± 7.46 b	76.24 ± 7.46 a

[1]Different letters denote significant differences between the untreated and the treated part of the leaf ($P < 0.05$). Means were separated by Student's *t*-test for paired means ($P < 0.05$).
[2]S.E.: standard error.

Table 6. Effect on fecundity, fertility (%) and sex ratio (%) in *Tetranychus urticae* females exposed to three concentrations of an ethanolic extract of *Chenopodium quinoa*.

Extract % (w/v)	n^1	Fecundity				Fertility		
		Average number of eggs/female[4] ± S.E.[3]			$n°E^2$	Fertility[4] (%)	Females (%)[5]	Males (%)[5]
		24 h	48 h	72 h				
Ethanol	9	7.88 ± 1.57 a	15.00 ± 3.08 a	19.88 ± 4.27 a	159	79.51 ± 5.40 a	64.62 ± 7.54 A	35.38 ± 7.54 B
2	8	7.10 ± 0.74 a	10.90 ± 1.65 a	13.50 ± 2.79 a	135	35.10 ± 11.47 b	56.99 ± 10.40 A	43.01 ± 10.40 A
3	10	5.44 ± 0.87 a	11.22 ± 1.99 a	15.11 ± 2.69 a	136	37.45 ± 8.39 b	64.07 ± 7.59 A	35.93 ± 7.59 B
4	10	7.40 ± 0.76 a	12.60 ± 1.81 a	15.10 ± 2.57 a	151	16.73 ± 7.99 b	53.46 ± 17.58 A	46.43 ± 17.58 A

[1] n: Initial number of females/treatment.
[2] $n°E$: Total number of eggs treated with 2, 3 and 4 % (w/v) extract and ethanol as control.
[3] S.E.: standard error.
[4] Means with a different lowercase letters within each column are statistically different according to the Tukey test ($P < 0.05$).
[5] Means with different uppercase letters in each row are statistically different according to Student's *t*-test for paired means ($P < 0.05$).

With respect to lethal time (Table 8) it can be seen that with increasing concentrations of the extract, the time needed to produce death in 50 % or 90 % of *T. urticae*, was reduced at the different concentrations. Thus, the lowest LT_{50} (12.3 h) was achieved with the highest concentration tested (10.5 %). Thus mortality rates were not an isolated factor, but directly related to the time required to produce such mortality.

DISCUSSION

Different extracts and oils derived from plants possess compounds that have shown lethal effects (to mobile stages and eggs) and sublethal effects (repellency, irritancy, effects on fertility and fecundity) against insects and mites (Isman 2006; Bakkali *et al*. 2008; Sertkaya *et al*. 2010). Deterrent effects of crude extracts of *C. quinoa* varieties ('markjo' and 'tarwi') have been reported for the tick *Boophilus microplus* (Canestrini) (Acari: Ixodidae) (Zegarra 2010). Currently, there are no previous studies on the activity of ethanolic extracts of *C. quinoa* on spider mites. Beyond the saponins and their known effects on various arthropods, the extract components of *C. quinoa* have not been identified and quantified, and their mode of action is unknown. The identification and quantification of saponins of *C. quinoa* have been studied in recent years (Woldemichael & Wink 2001; Zhu *et al*. 2002; Kuljanabhagavad & Wink 2009; Gómez-Caravaca *et al*. 2012). Lozano *et al*. (2012) determined that the total amount of saponins in an ethanolic extract obtained from peel grain quinoa was in the range of 47.3 % to 56.0 %. The high percentage of toxic saponins in the extract suggested the possibility that it might be used against mites because of an acaricidal effect. However, the possible synergistic action of other unknown, minor constituents, cannot be completely ruled out.

The work of Francis *et al*. (2002), Sparg *et al*. (2004) and De Geyter *et al*. (2012) indicate that the mode of action of saponins may be a cytotoxic effect causing disruption of the cell membrane as observed in haemocytes and other cell types. Another interesting component in the extract of quinoa seed husk is the presence of 20-hydroxyecdysone, a phytoecdysteroid that in many cases are much more active than natural moulting hormones, which in part has been attributed to their greater resistance to deactivation by the insect. These molecules cause

Table 7. Lethal concentrations ($LC_{50,90}$) of *Chenopodium quinoa* extract on adult females of *Tetranychus urticae*.

LC_{50}	CI 95 %	LC_{90}	CI 95 %	Slope		
				± S.E.	χ^2	d.f.
1.24	0.87–1.58	4.34	3.73–5.17	1.32 ± 0.19	43.81	1

Probit analysis (SAS Institute, 2001); LC: lethal concentration; CI: confidence interval; S.E.: standard error; χ^2: chi square; d.f.: degrees of freedom.

Table 8. Time (in hours) required for different concentrations of the ethanolic extract of *Chenopodium quinoa* to generate 50 % and 90 % mortality for adults of *Tetranychus urticae*.

Concentration	LT_{50}	CI_{lower}	CI_{upper}	LT_{90}	CI_{lower}	CI_{upper}	Regression line		
							a	*b*	R^2
1.5 % w/v	51.35 a	44.91	60.66	197.61 a	141.41	337.13	3.05	0.89	0.93
3.0 % w/v	33.96 b	29.01	39.38	166.90 a	119.48	287.03	16.71	0.86	0.95
4.5 % w/v	21.60 c	14.08	28.02	60.80 b	44.79	114.27	27.49	1.07	0.89
6.0 % w/v	16.51 c	13.56	19.17	55.01 b	47.29	67.24	38.39	0.89	0.88
7.5 % w/v	15.25 c	12.26	17.92	54.44 b	46.44	66.84	44.49	0.8	0.86
9.0 % w/v	15.36 c	13.03	17.47	38.05 c	33.72	44.2	45.48	0.89	0.74
10.5 % w/v	12.33 c	10.15	14.23	28.89 d	25.58	33.52	57.16	0.72	0.70

Lethal time ($LT_{50, 90}$) followed by the same letters in the same column are not significantly different based on the 95 % CI overlap (Vargas & Ubillo 2005); %: concentration of extract (% w/v); LT: lethal time; CI: confidence interval; *a*: constant; *b*: slope; R^2: coefficient of determination.

malformations, sterility and death of the insect (Marco & Tomás 1988).

Our results revealed a significant acaricidal activity of the ethanolic extract of *C. quinoa* against adults and nymphs of *T. urticae*. For adult females there were concentrations that reached mortalities higher than 50 % after 24 h and 90 % after 72 h. This corresponds with the results of Shi *et al*. (2006) who applied a chloroformic extract of *Kochia scoparia* (L.) (Chenopodiaceae) on *T. cinnabarinus* and obtained a mortality of 81.01 %. For nymphs the effect was greater than with adults, because at 24 h a mortality 99 % was achieved and 100 % at 48 h. One of the constituents present in the husks of *C. quinoa* is ecdysteroids, which have been isolated and identified by Zhu *et al*. (2001) and Kumpuna *et al*. (2011). It was found that the phytoecdysteroids can regulate gene activity, nucleic acid metabolism, protein synthesis, development, reproduction, and diapause in insects (Chow & Lu 1980). However, the most important function of these compounds is their moulting inhibitory activity, which has been extensively examined in research on insecticides (Karlson 1980). Bergamasco & Horn (1980) indicate that the five ecdysteroids present in the husks of quinoa are highly active, possibly causing a disorder in the normal development of several insect pests in feeding trials, and that it is therefore likely that the presence of these ecdysteroids contributes to the resistance of quinoa to insect attack. Thus the early and high mortality exhibited by nymphs of *T. urticae* is probably due to an incomplete moulting process which leaves individuals in an incomplete developmental stage. This might affect food intake and consequently lead to premature death.

The LC_{50} and LC_{90} values for adult females were 1.24 % and 4.34 % w/v, respectively. Wei *et al*. (2011) found an LC_{50} of 8.10 % w/v 24 h after having applied an ethanolic extract of *Aloe vera* L. (Asphodelaceae) on *T. cinnabarinus*.

The ethanolic extracts of *C. quinoa* did not show an ovicidal effect on *T. urticae*. Yanar *et al*. (2011) applied methanolic extracts of *Anthemis vulgaris* L. (Asteraceae) and *Lolium perenne* L. (Poaceae) on eggs of *T. urticae* and obtained mortalities of 25.38 % and 24.40 %, respectively. Erdogan *et al*. (2012) found that ethanolic extracts of *Rhododendron luteum* Sweet. (Ericaceae), *Helichrysum arenarium* L. (Asteraceae), *Allium sativum* L. (Amaryllidaceae), *Veratrum album* L. (Liliaceae) and *Tanacetum parthenium* L. (Asteraceae) had no ovicidal effect on *T. urticae* as there were no hatching problems. Although *T. urticae* eggs sprayed with ethanolic

extracts of *C. quinoa* had no problems in hatching, there was an effect on the viability of offspring, reaching between 17 and 35 % mortality. In contrast to the ethanol control (100 %), only 80 % of the offspring reached adulthood.

The extract of *C. quinoa* had a notable acaricidal effect when adult mites were exposed to its residue, achieving between 73 and 91 % mortality at 72 h after application. Similarly, Sivira *et al*. (2011) recorded 42 and 73 % mortality of *T. cinnabarinus* with 10 % ethanolic extracts of *Lippia origanoides* H.B.K. (Verbenaceae) and *Gliricidia sepium* (Jacquin) Kunth ex Walp. (Fabaceae) respectively, using the leaf disk immersion technique. Both species possess terpenoids, flavonoids, and saponins (phenolic groups).

Our study showed that the *C. quinoa* extract strongly repelled adult *T. urticae* adults, but lost the effect over time. Fernandes *et al*. (2010) also found a good repellency response by *Amblyomma cajennense* (Fabricius) (Acari: Ixodidae) when exposed to an ethanolic extract of *Chenopodium ambrosioides* L. (Chenopodiaceae). Moreover, various authors found significant repellency of terpenoids to tetranychid mites (Mansour *et al*. 1986; Tsolakis *et al*. 2002; Tsolakis & Ragusa 2004).

The sex ratio of *T. urticae* offspring appeared to have been affected by some of the applications of the ethanolic extract of *C. quinoa* on the female adults. The percentage of females was reduced and that of males increased so that the difference between female and male numbers was no longer significant, as in the control. The sex ratio obtained in control (ethanol), with females predominating, was similar to that recorded by different authors in the absence of acaricides (chemical or natural). Peralta & Tello (2011) found a female/male sex ratio of between 78 and 85 % on three muskmelon varieties (*Cucumis melo* L.) and Tello *et al*. (2009) recorded a sex ratio of 76.28 % females for the same species on *Dianthus caryophyllus* var. Celta. Cakmak & Demiral (2007), found a sex ratio for *T. cinnabarinus* on *Fragaria* × *ananassa*, ranging from 78 % to 85 %.

We conclude that compounds in *C. quinoa* have great acaricidal potential and this study lays a solid foundation for developing a *C. quinoa* based mite control product.

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